Gene Expression Profiling of Lung Tissue of Rats Exposed to Lunar Dust Particles

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Introduction

The purpose of the study is to analyze the dynamics of global gene expression changes in the lung tissue of rats exposed to lunar dust particles. Multiple pathways and transcription factors were identified using the Ingenuity Pathway Analysis tool, showing the potential networks of these signaling regulations involved in lunar dust-induced prolonged proflammatory response and toxicity. The data presented in this study, for the first time, explores the molecular mechanisms of lunar dust induced toxicity. This work contributes not only to the risk assessment for future space exploration, but also to the understanding of the dust-induced toxicity to humans on earth.

Materials and Methods

Lunar dust exposure and sample collection:
F344 rats were exposed for 4 weeks (6h/d; 5d/wk) in nose-only inhalation chambers to concentrations of 0 (control air), 2.1, 6.8, 21, and 61 mg/m^3 of actual lunar dust. Animals were euthanized at 14 weeks after the last inhalation exposure. After being lavaged, lung tissue from each animal was collected and snap-frozen in liquid nitrogen.

RNA isolation and Genearray analysis:
The total RNAs were isolated from the lung tissues. Four samples of each dose group were analyzed using Agilent Rat GE v3 microarray to profile global gene expression of a total 44K transcripts.

Data analysis:
Data processing and visualization- expression values were extracted using the GenePix Pro 7 software. After background subtraction, normalization, and log transformation, t-tests were used to compare the mean expression levels of each exposed group to the control group. Correction for multiple testing was made using the method of Benjamini, Krieger, and Yekuteli (1) controlling the false discovery rate to .025 (low, dose =2 case) or .05 (low, dose = 6, and both high cases). Genes with significant changes of at least 1.75 fold were identified as gene of interest.
The data were further integrated into the Ingenuity system to analyze the gene ontology (GO), pathway distribution and putative upstream regulators and gene targets.

Results

In general, the gene expression profiles in the lung tissues exposed to lunar dust exhibited significant changes. Lunar dust, at the dose as low as 6.8mg/m^3, resulted in prolonged large scale molecular changes, even though there was no significant pathological changes in the lung tissue.

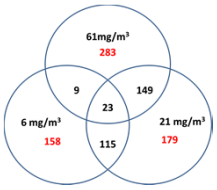
	2.1 mg/m^3	6.8 mg/m^3	21 mg/m^3	61 mg/m^3
Protein coded genes	6	305	466	464
Putative gene transcripts	24	511	546	301
Total	30	816	1012	765

Table 1. The number of genes with significant changes in different dose group

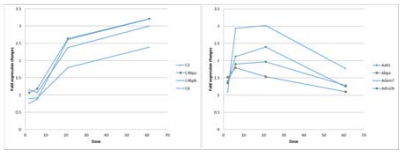
Dose dependent expression patterns of genes with significant changes

21 mg/m^3 dose group apparently shared 115 genes of significant changes with 6 mg/m^3 group and 149 genes with 61mg/m^3 group (Figure 1a). However, the sets of genes with significant changes in 6mg/m^3 group and 61 mg/m^3 group are distinct.

1a



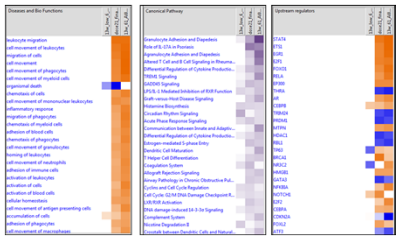
1b



The expression patterns of genes with significant changes are mostly dose dependent (Figure 1b). Data will be further analyzed for a panel of potential biomarkers for dust induced toxicity.

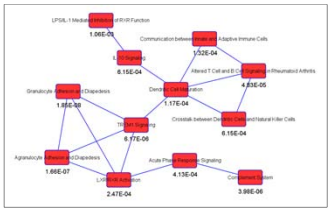
Different functions and pathways involved in the response to low dose and high dose dust

The comparison pathway analysis indicated that effects of low dose dust exposure are unique, broad, but much less toxic, compared with those of high doses (both 21 and 61mg/m^3, Figure 2). The activation of various pathways showed a dose dependent response.



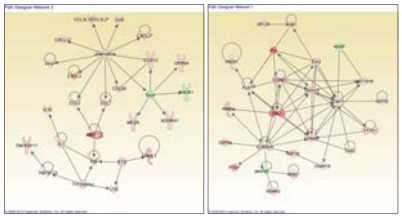
Top significant pathways identified in 61mg/m^3 dose group

More than 25 pathways were identified based on the set of significant genes changed in the lung tissue of rats exposed to the highest dose of 61mg/m^3. The top significant pathways are listed in Figure 3. These pathways are cross-linked and act as an integrated network.



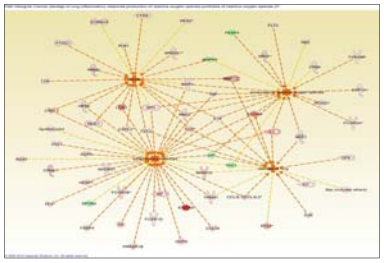
Direct gene interaction networks identified in 61mg/m3 dose group

Genes and encoded proteins are interacting with each other. Top identified networks are mainly involved in the process and control of cell cycle, immune cell trafficking, hematological disease, and cancer. In Figure 5, two networks, (left) cytokine-chemokine mediated immune cell movement and trafficking, and (right) cyclin mediated cell cycle controlling, are presented.



Significant function changes involved in disease progression identified in 61mg/m^3 dose group

Significant disease progressions and function changes are identified, especially in the 61mg/m3 dose group. In Figure 6, inflammatory response, production of reactive oxygen species, damage of lung, and cancer are presented with associated genes whose expressions are up- or down regulated.



Conclusions

- **Except for 2mg/m3 lunar dust exposure, the exposure to 6, 21, and 61 mg/m3 lunar dust caused dramatic global gene expression changes in the lung tissues. Most of the changes are dose-dependent.**
- **The responses of lung tissue to low dose lunar dust are distinguished from those of high doses, especially those after 61mg/m^3 dust exposure.**
- **Multiple pathways, functions, and upstream regulators have been identified in response to lunar dust induced damage in the lung tissue, which include inflammatory response, and potential induction of carcinogenesis.**
- **Our results can be used to uncover the mechanisms underlying dust-induced toxicity and pathological changes, as well as for identifying biomarkers and development of countermeasures.**

Reference:
Benjamini, Y., Krieger, A., and Yekutieli, D. (2006). Adaptive linear step-up procedures that control the false discovery rate. Biometrica 93: 491 – 507.

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